UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

PNW-2203-80-01

December 1979

FIELD EVALUATION OF BACILLUS THURINGIENSIS AND BACULOVIRUS FOR CONTROL OF THE WESTERN SPRUCE BUDWORM: AERIAL APPLICATION TRIALS

By

M. J. Stelzer and C. G. Thompson

U.S. GOVERNMENT PRINTING OFFICE : 1973 O—522-184

6200—M7 (10/73)

FIELD EVALUATION OF BACILLUS THURINGIENSIS AND BACULOVIRUS FOR CONTROL OF THE WESTERN SPRUCE BUDWORM: AERIAL APPLICATION TRIALS

Principal Investigator: M. J. Stelzer

Co-investigator: C. G. Thompson

Organization: PNW Forest and Range Experiment Station

APPROVALS:

C. G. Thompson, Project Leader	Date
Robert M. Romancier, Assistant Director	Date
Max W. McFadden, Program Manager	Date

FIELD EVALUATION OF BACILLUS THURINGIENSIS AND BACULOVIRUS FOR CONTROL OF THE WESTERN SPRUCE BUDWORM: AERIAL APPLICATION TRIALS

Prepared by

M. J. Stelzer

and

C. G. Thompson

December 1979

TABLE OF CONTENTS

	Page
OBJECTIVES	1
Specific objectives	1
JUSTIFICATION STATEMENT	2
METHODS	3
Proposed study areas	3
Treatments and design	3
Monitoring larval development	4
Application parameters	4
Monitoring spray deposit	5
Biological deposit assessment	6
Larval density sampling	7
Pupal sampling	8
Defoliation estimates	8
Long-term evaluation	9
COOPERATORS	10
LITERATURE CITED	12
SAFETY AND HAZARD ANALYSIS	14
BUDGET	16
FY-1980 Bt Aerial Trials	16
ADDENDUM TO BT STUDY PLAN: Virus Field Experiment FY-1980	18
Budget	21

OBJECTIVES

The overall objective of this study is to test and compare efficacies for the western budworm, Choristoneura occidentalis of aerial applications of three formulations of Bacillus thuringiensis. These tests are based on results of laboratory and ground tests conducted under the auspices of the CANUSA-West Program in 1979 and of aerial tests conducted against the eastern budworm, C. fumiferana in Canada and in the eastern U.S. from 1975 to 1979. It is anticipated that the results of our 1980 study will arrant pilot control (operational tests of a Bt formulation in 1981 and registration and operational use by 1982. It is expected that the study will generate enough information to allow a decision to be made as to whether either of the baculovirus treatments are capable of initiating epizootics.

Specific objectives are:

1. To determine the population reductions obtained with each treatment in the year of treatment and in the year(s) following treatment.

2. To determine the foliage protection provided by each treatment in the year of treatment and in the year(s) following treatment.

3. To evaluate the postreatment residual life of Bt spray deposits on foliage.

4. To determine the amount of disease-free budworm reinvasion or recovery in treated plots in the year(s) following treatment.

JUSTIFICATION STATEMENT

The development and evaluation of microbial agents for control of the western spruce budworm has been identified as a major objective of the spruce budworm RD&A Program. Because Bacillus thuringiensis and baculoviruses have little or no impact on non-target organisms, they can provide important alternatives to chemical insecticides.

Tripp (1972) reported population reductions of 96-99% following aerial application of Bt against the eastern spruce budworm on balsam fir. Aerial applications of Bt-chitinase in combination against the eastern spruce budworm provided good foliage protection (Dimond 1972, Smirnoff et al. 1973, Smirnoff 1974). Field efficacy of Bt was increased with the addition of small quantities of Orthene against moderate budworm densities, but did not provide satisfactory control against high popula- densities (Morris 1972). Cunningham et al. (1975), achieved population reduction of 84-91% on white spruce (Picea glauca) and of 21-65% on balsam fir (Abies balsama) with aerial applications of a nuclear polyhedrosis virus; however, there was no reduction in defoliation. Mist blower applications of Bt in Canada failed to provide satisfactory foliage protection (DeBoo and Campbell 1974).

While microbial agents have undergone extensive testing against the eastern spruce budworm, C. fumiferana little or no information is available for the western spruce budworm C. occidentalis under the

greatly differing conditions of climate, topography, and host type of western forests. In the west populations are most destructive on Douglas-fir, true fir species, and Engelmann spruce. This contrasts to the balsam fir-white spruce host complex in eastern U.S. and Canada.

METHODS

Proposed study areas:

We propose to conduct this trial on the Kaibab National Forest. Surveys during the Fall-1979 by Region 3, FIDM indicate an average of about 30 egg masses/M^2 branch area on ca. 90,000 acre of mixed fir type between Jacob Lake and North Rim Grand Canyon, Arizona. The population is described as entering the peak year of an outbreak cycle and should provide a vigorous population as a challenge to treatment efficacy. This area has good road access for sampling crews and is large enough to provide adequate buffer areas between all of the test plots.

Treatments and design:

The Bt treatments will consist of the following:

1. Thuricide-16B mixed 1:1 with water.
2. Thuricide-32B mixed 1:3 in 25% molasses.
3. Abbott ABG-6103 mixed 1:1 with water.

The 3 tank mixes will all be applied at a rate of 1 gpa, giving a dosage of 8 BIU/acre. Each treatment and an untreated check will be replicated 5 x on 100-acre sized plots in a completely randomized design. Plot corners will be marked with "day-glo" colored panels to facilitate the precision of applications. Cross-bows will be used to shoot monofilament lines over tree-tops to raise the colored panels. These marking will be supplemented by balloons where necessary.

Monitoring larval development:

Prespray insect development will be monitored to facilitate application timing. Plots will be considered ready for spray when the average larval instar is $\geq$ 4.0 (4th instar). Larval sampling for instar developmental analysis will begin after bud burst (shoot elongation). Larvae on 2 branches from 10 trees/plot will be consolidated. Head capsules measurements for instar determination will be made on at least 50 randomly selected larvae and the average instar calculated.

We will attempt to treat an equal number of plots having similar insect development with each of the 3 Bt treatments on a given day: Assignment of treatments and order of spraying will be made as soon as the plots reach the designated developmental stage, i.e. $\geq$ 4.0 instar.

Application parameters:

Treatments will be applied by fixed-wing aircraft using standard boom and nozzle system. The spray aircraft will be calibrated to deliver 1 gallon of each spray mixture/acre in droplets having a volume-median diameter (vmd) in the range from 250-300 µm. The spray aircraft will be characterized with all 3 tank mixes well in advance (before May) of actual treatment timing.[1] Composition of tank mixes for 100 gallon quantities is presented in the following table.

---

[1] Tentative plans have been discussed between the principle investigator and R-3, FIDM for joint calibration tests in R-3 in spring-1980. Spread factor values of 1.88, 1.99, and 3.11 will be used in the calculation of vmd for the Thuricide-16B, Thuricide-32B, and ABG-6103 tank mixes, respectively.

Product	Quantity/100 gallons[1]		
	Bt concentrate	Molasses	Water
Thuricide-16B	50 gal	--	50 gal
Thuricide-32B	25 gal	25 gal	50 gal
ABG-6103	25 gal	--	75 gal

[1] Rhodamine B extra S dye for deposit assessment added to tank mixes at rate of 0.83 lbs/100 gal (.1% w/v).

All spraying will be done during early morning hours as long as wind speeds are $\leq$ 6 mph and temperature inversions are maintained.

Monitoring spray deposit:

Kromekote cards will be used to estimate vmd and droplet density of deposits on the target plots. Assessment points will be established at 15 points along a transect perpendicular to the direction of swath applications, to pass thru the general location of the trees selected for efficacy evaluations. Each station will have 2 cards mounted on a wire holder (30 cards/plot). Under current plans having plot dimensions of 1400 x 3150', and flying the long axis, a station will be placed at each border and at 13 in-plot locations at 100' intervals.

Adjustments will be made so that station locations coincide with the largest canopy opening available in the immediate area.

Biological deposit assessment:

A biological assessment of Bt spray deposits will also be conducted. Two mid-crown branches (6-10" long) will be removed from 15 tagged trees/plot at postspray intervals of 1-4 hours, 3-day, 5-day, and 10-14 days. A total of 5 tips of current foliage will be removed from each of the 2 branch sample/tree. The tips will be put singly in to 100ℓ petri dishes containing one 4th instar larvae. Mortality readings and diagnoses for causal agent will be made after 7 days, and a percent Bt infection calculated for each tree (15 trees/plot/sample timing).

Foliage bioassays will be conducted on at least 1 plot representing each of the 3 Bt treatments and the check. Expansions to include additional plots will be governed by resource availability.

This is an exploratory study to evaluate the potential of this technique in deposit assessment, and it may be possible to relate the results to post-treatment larval population reductions.

The percent Bt infection rates will be analyses as for a 4 x 4 factorial (4 treatments at 4 sample intervals) in a completely random design with 15 replicates/treatment-timing combination. The ANOVA is as follows:

SOURCE		df
Treatments		15
Sample timing	3	
Bt-Formulation	3	
Timing x Formulation	9	
Error		224
TOTAL		239

Larval density sampling:

Spruce budworm larval population densities will be determined at 24-48 hours prespray and at 14 days post-treatment on 15 trees designated for sampling on each plot. The sampling procedure is a modification of that described by Carolin and Coulter (1972). A pole pruner fitted with a collecting basket will be used to clip 38 cm branch samples in mid-crown. A total of 2 branches/tree prespray and 4 branches/tree post-spray will be collected from each tree. The branch samples from each tree will be combined and bagged for transport to a central counting station. Budworm larvae will be removed from the branch samples by the "drum beating" technique and counted. In addition, the number of buds will be counted and the area of each branch determined (length x width divided by 2). Larval density will be expressed both as the number of budworm larvae/meter^2 branch area and per 100 buds on each tree. Population reduction (PR) values will be computed at each tree as follows:

$$PR = \frac{\text{Prespray density - Postspray density}}{\text{Prespray density}}$$

These values will be the response variables used in data analyses using the prespray density as a covariate.

The ANOVA table for 14-day population reduction percents will be

SOURCE	df
Treatment	3
3 Bt treatments vs check	1
2 thuricide vs ABG-6103	1
Thuricide-16B vs T-32B	1
Error	16
TOTAL	19

Pupal sampling:

Since no proven method now exists for sampling pupal density on an area basis, this stage will be sampled to measure pupal quality including:

1. Rates of parasitism.
2. Rates of microbial infection.
3. Survival rates.
4. Sex ratios.
5. Pupal weight by sex.
6. Adult weight by sex.

Pupae will be collected by hand picking and/or branch sampling by polepruner. We will attempt to collect at least 100 pupae/plot, the pupae coming from at least 10 or more different trees. For convenience the pupae will be held in groups of 10 during rearing to the completion of adult emergence.

Sublethal doses of Bt are reported to lower pupal weights and adult fecundity. Results of the quality measurements will be inspected for trend differences on treated and check plots. However, no statistical tests are planned at this time.

Defoliation estimates:

A modification of the procedure developed by FIDM will be used to assess feeding damage. This method involves the examination of 25 shoots per branch, and classifying each shoot as to the amount of new foliage consumed or destroyed within 6 classes: 1=0%; 2=1-25%; 3=26-50%; 4=51-75%; 5=76-99%; and 6=100%.

The frequency number in each class interval will be multiplied by the mid-class value and the resultant subtotals then totalled and divided by 100 (= number on shoots examined) to give a defoliation value for each tree.

Plot averages will be analyzed as for a completely random design having 5 replicates (plots) and 4 treatments. The analysis of variance table will be

SOURCE	df
Treatment	3
Error	16
TOTAL	19

Tukeys-w procedure will be used to compare the treatment means at the 05 level.

Long-term evaluation:

The experimental plots will be visited the year following treatment. Larval population density samples, on the same tagged trees will be taken. The timing of this sample will coincide with the timing of the prespray sample during the year of spraying. The population density values between the 2 years will be compared with (time) years a split-plot treatment.

COOPERATORS

This study will be conducted in cooperation with Region 3, FIDM, Albuquerque, New Mexico. Overall responsibility for the direction of the project rests with the principle investigator. However, certain aspects in which Region 3, FIDM will assume the lead role are identified. These will include:

1. Arrangement and conduct of aircraft calibration trials of the 3 microbial formulations (early spring, 1980) to insure that the aircraft and spray system can meet application parameters designated in the Study Plan.

2. Recruitment and hiring of 10 GS-4 temporary helpers (program funded) for a period not to exceed 7 weeks with the duty station at Jacob Lake. Employment to start on or about June 1 or beginning of spruce budworm larval activity.

3. Monitor day-degree accumulations above base $42^{o}F$ in general study area, e.g. Jacob Lake or Fredonia Ranger Station.

4. Prepare and let contract application aircraft and provide necessary Forest Service personnel required at airport during operations.

5. Provide assistance during location and marking of spray plots (20 plots x 100 acre/plot).

6. Work with principle investigator in development of a public awareness program and provide liaison with the Forest Supervisor's Office and staff.

Technical input concerning spray formulations is being provided by representatives of Sandoz, Inc., San Diego, California and Abbott Laboratories, Chicago, Illinois.

LITERATURE CITED

Cunningham, J. C., Kaupp, W. J., McPhee, J. R., Howse, G. M., and Hernden, A. A. 1975. Aerial application of nuclear polyhedrosis virus on spruce budworm on Manitoulin Island, Ontario in 1975. Review of App. Entomol. 64:1905.

DeBoo, R. F. and Campbell, L. M. 1974. Assessment of effectiveness by mist blower and aerial application, Spruce Woods, Manitoba. In: Bacillus thuringiensis. Evaluation of commercial preparations of Bacillus thuringiensis with and without Chitinase against spruce budworm. Inf. Rep. CC-X-59: C-1-31.

Dimond, J. B. 1972. A demonstration of Bacillus thuringiensis plus the enzyme chitinase against the spruce budworm in Maine. Maine Agric. Exp. Stn., Misc. Rep. 144:26p.

Morris, O. N. 1977. Long-term study of the effectiveness of aerial application of Bacillus thuringiensis-acephate combinations against the spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae). Can. Entomol. 109:1239-2148.

Smirnoff, W. A., Randall, A. P., Martineau, R., Haliburton W., and Junesu, A. 1973. Field test of the effectiveness of chitinase additive of Bacillus thuringiensis Berliner against Choristoneura fumiferana (Clem.). Can. J. For. Res. 3:228-236.

Smirnoff, W. A. 1974. Three years of aerial field experiments with Bacillus thuringiensis plus chitinase formulation against the spruce budworm. J. Invertebr. Pathol. 24:344-348.

Tripp, H. A. 1972. Field trials to control spruce budworm Choristoneura fumiferana (Clem.), through aerial application of Bacillus thuringiensis. Proc. Entomol. Soc. Ont. 103:64-69.

SAFETY AND HAZARD ANALYSIS

Inasmuch as all Bt products are biological materials they do not have the physico-chemical properties of chemical insecticides. Extensive safety testing has been accomplished on Bt. For example, 18 humans ingested 1 g of Bt daily for 5 days. Complete physical and laboratory examinations were given prior to the experiment, at the end of the 5 day ingestion period, and 4-5 weeks later. All subjects remained well during the course of the experiment, and all laboratory findings were negative.

Results of a number of Bt animal safety tests are summarized in Table 1.

Table 1. Bt animal safety tests.

Test animal	Type of test	Dose	Response
Rat	Acute Mammalian	24 g	negative
Mice	Acute Mammalian	10 g/Kg	negative
Dog	Acute Mammalian	400 mg/Kg	negative
Albino rabbit	Dermal	50 mg	local, mild erythema only
Mice	Inhalation	10 g @ 4X	negative
Albina rabbit	Eye Irritation	0.1 ml of a 20% suspension	slight reddness of eyelid at 3h and 24 h Clear at 48h

Avian and fish toxicity studies have been conducted with several Bt preparations. Laying hens, chicks, pheasants, partridge, and bobwhite quail fed Bt showed no differences in behavior, growth rates, or pathology to the control animals. Tests employing rainbow trout and bluegills showed no deaths or symptoms of alimentary or behavior disturbances following exposure to Bt. In a test with juvenile Coho salmon (1.6" in length) Bt was about 1/30 as toxic as DDT.

Human and animal safety tests with numerous baculoviruses provided results similar to those reported with Bt. In general, no changes in the health of the subjects under exposure was noted.

BUDGET
FY-1980 Bt Aerial Trials

		RWU-2203	CANUSA
a.	Manpower-F.S. Salaries		
	Stelzer	31,700	
	Thompson	11,500	
	Clark	12,100	
	Robinson } YACC	NC	
	Pietsch }	NC	
	Scott		
b.	Temporary help		
	Field Crew, 6 GS-4, 7 weeks @ 4.83/hr		8,100
	Lab Crew, 4 GS-4, 7 weeks @ 4.83/hr		5,400
	Overtime, 100 hrs total		500
		SUBTOTAL	14,000
c.	Per diem		
	PNW-2203 personnel, 310 days @ $16/day		4,960
	Lodging, 3 cabins, 2 months @ $450/mo		2,700
		SUBTOTAL	7,660
d.	Travel (mileage)		
	Air. Corvallis - Arizona & return (2 trips)		600
	Project Van (WCF)		300
	Rental pickups		
	4 @ $12/day for 8 weeks		2,688
	Gasoline		1,400
	(1981 Follow-up -- 2 men, 1 week)		(2,000)*
		SUBTOTAL	4,988
e.	Contracts		
	Aircraft, 1500 acres @ $10.00/acre		15,000
	Calibration-characterization of aircraft		500
	Analysis 500 spray deposit cards		750
	Rental of lab. space, 2 mo		500
	Statistical analysis, computer time		500
	(PNW furnishes salary of Biometrician)		
		SUBTOTAL	17,250

*Costs not included in 1981 Follow-up Subtotal.

BUDGET
FY-1980 Bt Aerial Trials
(continued)

		RWU-2203	CANUSA
f.	Supplies		
	Petri dishes		3,000
	Larval production & diet		600
	Plot markings-equip. & supplies		750
	Abbott ABG-6103 (600 acre-dose)		3,000
	Thuricide-16B (" " ")		3,000
	Thuricide-32B (" " ")		3,000
	Molasses		200
	Marker dye	500	
	Miscellaneous supplies		1,000
		SUBTOTAL	14,550
g.	Meetings		
	CANUSA		500
	Cooperators		500
		SUBTOTAL	1,000
	SUBTOTALS	55,800	59,448
	OVERHEAD	14,508	15,456
		70,308	74,904

ADDENDUM TO BT STUDY PLAN

Preliminary Efficacy Trial with Aerial Applications of Virus

M. J. Stelzer and C. G. Thompson

OBJECTIVE

To determine if an epizootic can be initiated by aerial application of a NPV and GV virus and carried over from one year to the next, i.e., do we need to achieve "insecticidal control" with the treatment or will a "partial take" provide long-term controls?

METHODS

Experimental plots:

Two plots of 100 acres in size will be designated for treatment (1-NPV, 1-GV). The 2 plots will be located (with adequate buffers) in the same general area as the Bt trial and one of the check plot from that study (nearest) will serve as a control.

Virus formulation and dosage

Both viruses will be applied at a dosage of 100 billion polyhedra/acre in 1 gallon of a tank mix containing 25% molasses and 0.5 lbs Shade^{R} UV protectant. Treatments will be applied with the same aircraft

used to apply the Bt treatments and following the same application parameters (250-300 μm vmd). Timing will coincide with peak 4th instar as monitored by periodic larval sampling after bud burst (see Bt Study Plan for details).

Efficacy measurements:

Population density measurements, collections of larvae for laboratory rearing, and defoliation evaluations will all be taken as described in the Bt Study Plan. Population density samples will be taken prespray at 7 and 14 days. Pupal collections will also be taken for quality analyses, postspray on 15 tagged trees/plot. The larvae on the branch samples will be collected and reared at the field laboratory to determine virus incidence levels at each timing.

The values obtained in these evaluations will provide the response variables for statistical analyses, including (a) population reductions at 7 and 14 days, (b) incidence of infection by NPV and GV, and (c) percent defoliation. The ANOVA Table will be:

SOURCE		df
Treatments		2
NPV & GV vs check	1	
NPV vs GV	1	
Error		42
TOTAL		44

Long-term assessment

A major objective of this study is to evaluate the long-term impact of the virus treatments. This will require at least a larval density sample shortly after bud burst in 1981. In addition, collections of larvae will be reared on diet to look for treatment variations in rates of virus infection, parasitism, pupal quality, and defoliation.

Treatment assessment at 2 years after treatment will be determined on the basis of projected population densities and virus infection levels in the surviving populations.

BUDGET
VIRUS FIELD EXPERIMENT
FY-1980

		CANUSA
a.	Salaries	
	Scott -- GS-9 Term Appt.	17,850
	Edwards -- GS-4 insectary man (for all RWU-2203 budworm needs including all bioassay insect requirements of the Bt field experiment)	10,049
b.	Virus production supplies	3,000
	Field rearing supplies: dishes	300
	diet	50
c.	Aircraft (200 Acres @ $10.00/acre)	2,000
	Molasses (100 gal)	100
d.	Long-term evaluations	
	1981	(2,000)*
	1982	(2,000)*
		$33,349
	OVERHEAD	8,671
	TOTAL	$42,020

*Costs not included in FY-1980 budget Total.

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

PNW-2203-80-02

December 1979

ADDENDUM TO FY 1979 STUDY PLAN

GROUND APPLICATION TRIALS[1]

By

M. J. Stelzer

[1]Field Evaluation of Bacillus thuringiensis and Baculovirus for Control of Western Spruce Budworm: Ground Application Trials.

U.S. GOVERNMENT PRINTING OFFICE : 1973 O—522-184

6200—M7 (10/73)

ADDENDUM TO FY 1979 STUDY PLAN

GROUND APPLICATION TRIALS[1]

Principal Investigator: M. J. Stelzer

Organization: PNW Forest and Range Experiment Station

APPROVALS:

C. G. Thompson, Project Leader	Date
Robert M. Romancier, Assistant Director	Date
Max W. McFadden, Program Manager	Date

[1] Field Evaluation of Bacillus thuringiensis and Baculovirus for Control of Western Spruce Budworm: Ground Application Trials.

TABLE OF CONTENTS

	Page
OBJECTIVES	2
METHODS	2
Population evaluations	2
Population quality	4
COOPERATORS	4
BUDGET	5

ADDENDUM TO FY 1979 STUDY PLAN
GROUND APPLICATION TRIALS[1]

M. Stelzer

Ground applications of Bacillus thuringiensis (Bt) provided near complete control (>95%) of spruce budworm larvae at 14 days after spraying. The trials were conducted in campgrounds along the Gallatin River South of Bozeman, Montana in 1979.

In addition to Bt we tested two baculovirus isolates for efficacy. Treatments were applied against late 5th instar larvae. Population reductions at 14 days using a nucleopolyhedrosis virus (NP) isolated from the European budworm, Choristoneura murinana, in a tank mix containing 2×10^9 polyhedra/gal, average 54.8% compared to 31.2% on untreated trees. Spruce budworm showed no response to a granulosis virus (GV) isolated from C. viridis with population reductions of 16.2 and 18.6% on treated and check trees, respectively. These results with the baculoviruses are not unusual in view of the very late timing of application and the short posttreatment interval (14 days). The incubation period for both viruses, under optimum conditions in the laboratory, exceeds 8 days. That over 75% of the population treated with NP had pupated by 14 days attests to the lateness of timing.

[1]Field Evaluation of Bacillus thuringiensis and Baculovirus for Control of Western Spruce Budworm: Ground Application Trials.

A follow-up on 1979 ground trials in 1980 is necessary to fully evaluate the results of these trials.

OBJECTIVES

The objectives of the follow-up observations will be to: (1) determine the extent of reinvasion by spruce budworm on the trees where larval populations were controlled with Bt in 1979, (2) carry-over population levels on the untreated trees, (3) to determine the carry-over, if any, of pathogens on the treated trees, especially of the baculoviruses, and (4) to make a damage appraisal of the 1979 treatments and degree of tree recovery.

METHODS

Population evaluations:

Trees sampled in 1979 have been mapped and tagged so they can be resampled in 1980. Trees will be sampled to estimate larval density as in 1979. Time of sample should coincide with the 1979 prespray sample (late June), as estimated by the accumulation of 200 day-degrees (base 42^{o} F). Two mid-crown branches will be taken from each tree and both the number of spruce budworm larvae/1000 in^2 branch area and/100 shoots (buds) calculated. These values will provide the response variables for analysis at each of the 3 campgrounds as follows:

A. Squaw Creek and Swan Creek. CRD. 10 reps/treatment.

SOURCE		df
Treatment		2
	16B + 32B vs check	1
	16B vs 32B	1
Error		27
	TOTAL	29

B. Red Cliff. 2 x 2 x 2 Factorial, CRD, 10 reps/treatment (2 dosages, 2 timings, 2 host species).

SOURCE		df
Treatment		7
	Species	1
	Dose	1
	Timing	1
	S x D	1
	S x T	1
	D x T	1
	S x D x T	1
Error		72
	TOTAL	79

Population quality:

Larvae occurring on the branch samples taken for density estimates will be collected for rearing on diet. A maximum of 10 larvae/tree will be reared individually in 1000 petri dishes to adulthood or death. Dead larvae will be diagnosed to determine the causal agent. Rearing results will be used to calculate rate of (a) parasitism; (b) infection by pathogen i.e., Bt, NPV, GV, other; (c) pupal-adult survival; and (e) sex ratio.

It is reasonable to expect the Bt and baculovirus treatments might affect all of the response variables being examined. In some cases no response would be expected, e.g., infection by Bt on check trees. Therefore, these data will be tabulated to examine means for trends or patterns before statistical analyses are conducted.

COOPERATORS

This is a cooperative study. The Principle Investigator (PI) is M. Stelzer, (PNW RWU-2203). Assistance in obtaining the necessary samples will be provided by the Spruce Budworm Program representative L. Stipe, Region-1, FIDM. Collecting supplies and data forms will be supplied by the PI; polepruners, paper bags, and a vehicle by R-1. This work will require 5 work days at the study site. Larval collections for rearing will be sent to the FSL Corvallis.

BUDGET

Air Travel (PI to and from Study Area)	175.00
Vehicle rental and mileage	400.00
14 days per diem (PI and Cooperator)	490.00
TOTAL	$1,065.00